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MICROBIOLOGICAL BURDEN ON THE SURFACES OF THE AIMP SPACECRAFT

PART 3

JULY 1966

GODDARD SPACE FLIGHT CENTER

GREENBELT, MARYLAND

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SUMMARY

Decontamination of the AIMP spacecraft has progressed through six phases of the assembly. The decontamination and assembly will be completed at the John F. Kennedy Space Center in Florida.

The decontamination procedure has reduced the microbial population on the prototype and flight spacecraft by approximately 2 logs. On the basis of data obtained thus far, it has been estimated that the total microbial burden of the spacecraft will be in the order of 1×10^5 organisms or less at liftoff.

Air sampling of the laminar crossflow room in which the spacecraft was assembled indicated that the air contained less than 1 viable particle per cubic foot of air per hour. Counts obtained from the air of a downflow room, also used for assembly of the spacecraft, indicated that contamination in the air was an order of magnitude lower than that detected in the crossflow room; the range was 0 to 0.08 viable particles per cubic foot of air per hour.

Microbial fallout on stainless steel strips and tryptic soy agar (TSA) plates was also studied for a 14-day period during the sixth assembly phase.

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INTRODUCTION

The decontamination and monitoring of the occluded surfaces of the AIMP spacecraft (surfaces under the protective cover) was completed before the spacecraft was shipped to the Eastern Test Range at Kennedy Space Center in Florida for final testing and assembly

This report, which describes the sixth phase of the assembly, is the third in a series on the decontamination and microbiological monitoring of the AIMP spacecraft. These reports follow the state of decontamination and microbiological contamination from the first to the final stages of assembly. A summary of the microbial contamination on the surfaces of the spacecraft during each phase of the assembly is reported below. For more detailed information of the first 5 phases of assembly see Parts One and Two (References 1 and 2). Decontamination and Monitoring during the sixth assembly phase extended from May 2, 1966 to May 18, 1966.

Decontamination and monitoring of the AIMP spacecraft for microbial contamination is in compliance with the NASA Spacecraft Decontamination Policy as stated in Management Manual 4-4-1 for decontamination of lunar landing hardware.

MATERIALS AND METHODS

Surfaces Sampled for Microbial Contamination

Table 1 lists the parts of the spacecraft sampled during the sixth phase of the assembly. Part 2 of this report (Reference 2) describes assembly phases one through five. The various areas of the AIMP spacecraft are classified with respect to the manner in which they are occluded by other parts of the spacecraft or exposed to the environment. The areas are classified as follows:

A. Interior surface areas of module frames, including walls, cavities and electronic circuit boards, but not including electronic components

Table 1

Parts of AIMP Samples for Microbial Contamination

Assembly Phase 6

Area		Surface Area (sq. in.)	Area Sampled (sq. in.)
A	GSFC fluxgate A/D electronics	151	4
	GSFC fluxgate electronics	151	4
	Optical aspect computer	151	4
	Optical aspect sensor	58	4
	Prime converter	164	4
	Programmer No. 1 (undervoltage)	136	4
	Solar-array regulator	66	4
	MIT plasma probe	335	4
	Antenna hybrid	162	4
	Telemetry encoder	133	4
	Range and range-rate No. 2	153	4
	Range and range-rate No. 3	105	4
	Univ. of California ion chamber	141	4
	Ames signal processor	143	4
	Univ. of Iowa particle detector	171	4
	Programmer No. 2 (IV stage timers)	135	4
B	C-frame occluded by circuit modules	448	32
B	Platform top covered by module frame	384	16
C	Top of module stack	328	16
C	Front face of stack	460	16
C	Inner surface of cover	1430	28
A	Turn-on and ordnance plug	83	4

Table 1

Parts of AIMP Samples for Microbial Contamination

Assembly Phase 6

(Continued)

Area		Surface Area (sq. in.)	Area Sampled (sq. in.)
C	Struts, C-frame to center tube	156	6
C	Center tube from platform to cover	256	8
C	Back of C-frame	450	8
C	Antenna cups below cover	36	36
C	Antenna supports	20	20
C	Yo-yo despin connector bracket	14	14
D	Center tube interior	340	8
D	Sun shield plate	34	4
D	Sun shield support rod	9	9
D	Fourth-stage spring assembly housing	20	10
D	Fourth-stage microswitch assembly	39	13
D	Fourth-stage flyaway connector box	11	11
D	Battery bracket and connector assembly	7	7
D	Battery	207	8
D	Spring seat assembly	85	18
D	Third-stage separation microswitch assembly	54	27
D	Lower cone	27	4
D	Center tube under lower cone	64	4
D	Platform under lower cone	65	4
E	Top cover	1369	4
E	Platform lower surface	400	32

B. Occluded surfaces obstructed by module frames, excluding exposed surfaces of the stacks of module frames

C. Inner surfaces of spacecraft occluded by protective cover

D. Interior surfaces and volumes of the spacecraft:

1. Body
2. Motor, volume of propellant
3. Assembly-occluded surfaces

E. Exterior surfaces of spacecraft

F. Volumetric components:

1. Internal electrical components
2. Retrorocket propellant

Two electronic circuit modules from each facet were sampled individually before and after decontamination. Half the total number of circuit modules were sampled. After they were stacked in the spacecraft, the top and face of alternate stacks were sampled.

Seven samples were taken from the inner surface of the cover, by sampling the sides of alternate facets and by taking three samples from the inside top of the cover. Exterior surfaces of the cover will be sampled just before launch. The exterior cover is now protected by a strip coating which will be removed a few hours before flight.

Half of all identical parts were sampled, and an attempt was made to sample all representative surfaces. In addition to surfaces of the flight spacecraft, the same surfaces of the prototype spacecraft were sampled.

Sampling Procedures

Same as previously reported (Reference 1)

Decontamination Procedure

Same as previously reported (Reference 1)

Media

Tryptic soy agar (Difco) was used for plate counts of all samples. Stainless steel strips were suspended and shaken in 1-percent Bacto peptone (Difco).

Incubation

Same as previously reported (Reference 1)

Air Sampling

Air samples were collected twice a day, in the morning and afternoon, during the 2-week period in which the prototype and flight spacecraft were being decontaminated and monitored. Sampling devices used and rooms monitored were:

Air sampler — A Reyneir slit sampler with a 1-hour clock was used. Air was drawn into the sampler at a rate of 1 cubic foot per minute for 60 minutes. Glass plates (150 × 20 mm) containing 60 ml of tryptic soy agar (TSA, Difco) were used in the sampler. One Reyneir sampler was used in each type of room.

Microbial Fallout — Microbial fallout was determined in two ways:

1. Stainless steel strips — Sterile, type 304 stainless steel strips, 1 by 2 by 0.06 inches, were exposed next to the Reyneir air sampler in each room. The strips remained undisturbed for 3 days during which the rooms were unoccupied. Thereafter, five strips were aseptically picked up daily in the late afternoon. Each strip was transferred to a screw-cap jar containing 50 ml of 1-percent Bacto Peptone and mechanically shaken for 5 minutes. Five-ml aliquots were plated out in duplicate from each jar. Strips were cleaned and sterilized as previously reported (Reference 3).

2. Settling plates — Five TSA plates were randomly spread out on the tables in the work area of each room. Plates were exposed twice daily, in the morning and afternoon, for 20 minutes. When the rooms were occupied, personnel worked over and around the exposed plates. The surface area of each petri plate was 9.6 sq. inches.

Rooms monitored

1. Crossflow room — Decontamination and assembly of the spacecraft was accomplished in a class 100 laminar crossflow room approximately 20 feet long, 15 feet wide, and 8 feet high. The Reyneir air samplers and the stainless steel strips were placed side by side in the center of the room, approximately

8 feet downstream of the spacecraft and the personnel. The room was occupied by a maximum of three people at any one time.

2. Downflow room — The spacecraft was stored in a portable class 100 laminar downflow room used for some decontamination and assembly. This room, with transparent, flexible walls, is 12 feet long, 8 feet wide, and 12 feet high. The Reyneir air sampler was placed as near the center of the room as possible without interfering with the spacecraft. The stainless steel strips were placed on a table near the air sampler and spacecraft. A maximum of three people occupied the room at one time.

3. Potting room — The potting room, a conventional clean room (class 10,000) used for potting and foaming of electronic circuit modules, is 8 feet long, 7 feet wide, and 9 feet high. The room was usually occupied by one person and, because of its small size, seldom more than two people at a time. The Reyneir air sampler and stainless steel strips were placed side by side on the bench top in the general work area.

RESULTS

Table 2 lists the microbiological contamination recovered from various surfaces of the AIMP prototype spacecraft before and after decontamination. The viable microbial population was reduced by approximately 2 logs per square foot.

Table 3 lists the microbiological contamination recovered from various surfaces of the AIMP flight spacecraft. The level of contamination "before" decontamination was much lower than that recovered from the prototype spacecraft (Table 2). The higher counts from the prototype were probably due to the fact that it was handled in a routine fashion without taking special precautions to keep it clean; i.e., it was not handled aseptically or stored in a clean room and it had not previously been cleaned or decontaminated. The flight spacecraft, on the other hand, had been decontaminated several times during assembly although only occluded surfaces were sampled each time (References 1 and 2). The viable microbial population "after" decontamination, however, was reduced to practically the same level on both spacecraft. This tends to indicate that the decontamination effort was reproducible and/or consistent.

Table 2

Microbial Contamination on Surfaces of AIMP Prototype Spacecraft

	average number of organisms per ft^2	
Occluded Section	Before Decontamination	After Decontamination
A	412	39
B	3366	36
C	1905	29
D	1225	53
E	2064	18
Average/ft^2	1794	35

Table 3

Microbial Contamination on Surfaces of the AIMP Flight Spacecraft

	average number of organisms per ft^2	
Occluded Section	Before Decontamination	After Decontamination
A	1152	36
B	234	28
C	228	33
D	307	39
E	162	28
Average/ft^2	416	33

Table 4

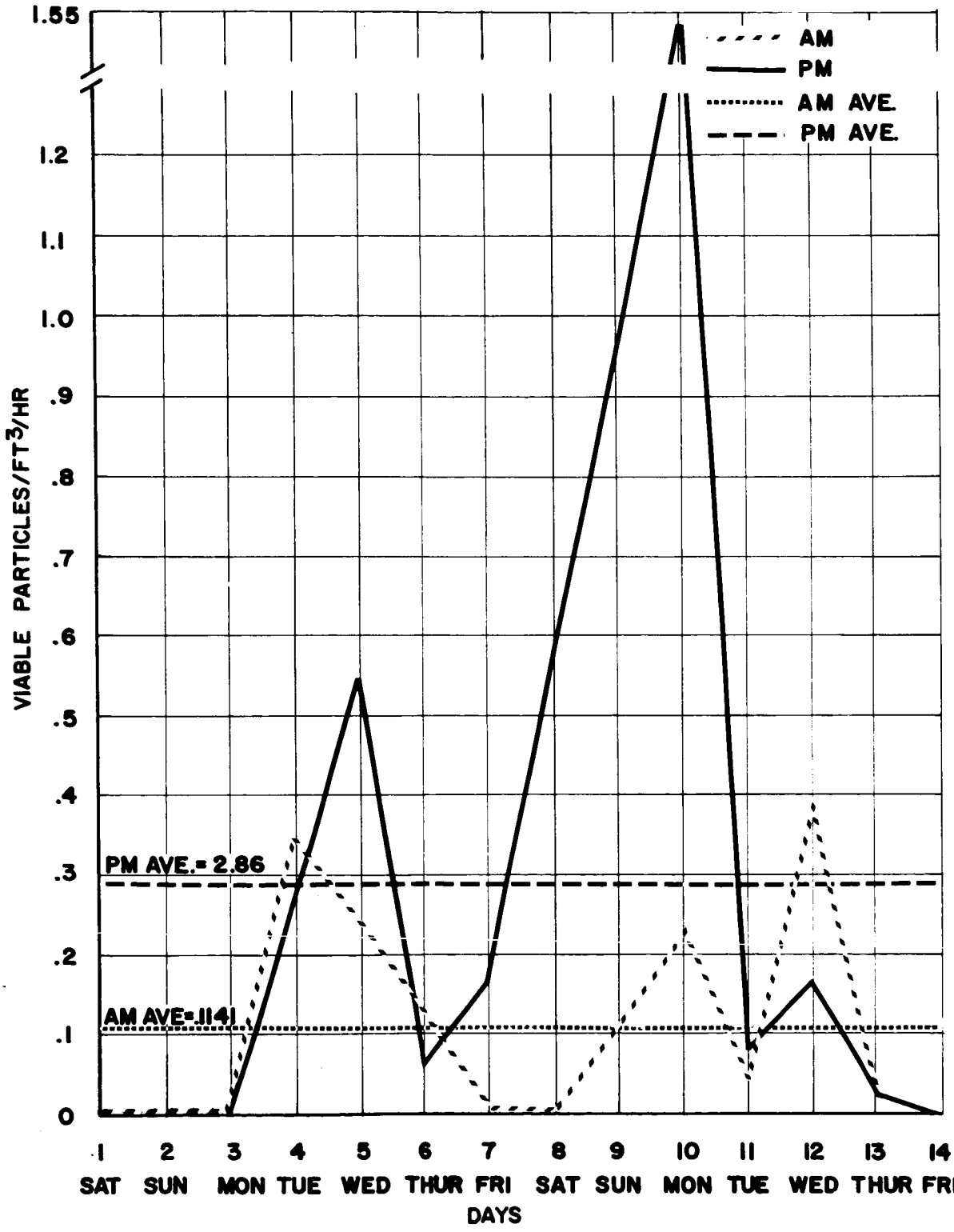
Summary of Microbial Contamination on
Surface of AIMP Flight Spacecraft

Assembly Phase	Date	average number of organisms per ft^2	
		Before Decontamination	After Decontamination
1	11-30-65	21,174	563
2	12-13-65	22,388	230
3	12-23-65	1,000	75
4	1-3-66	2,125	90
5	2-7-66	1,299	219
6	5-2-66 - 5-18-66	416	33
Average/ft^2		8,060	201
Average/500 ft^2		4,030,000	100,500

Table 4 summarizes the data obtained during the first six assembly phases (See References 1 and 2 for details of assemblies one through five). With the exception of assembly phases five and six, there was a 2-log reduction in the microbial population "after" decontamination. The low level of contamination "before" decontamination in assembly phase six was undoubtedly due to the fact that many of the surfaces had previously been decontaminated. Storage of the spacecraft in a laminar downflow clean room also kept, decontamination to a lower level. Consequently, the reduction "after" decontamination was not as great as previously achieved, but was still greater than a log.

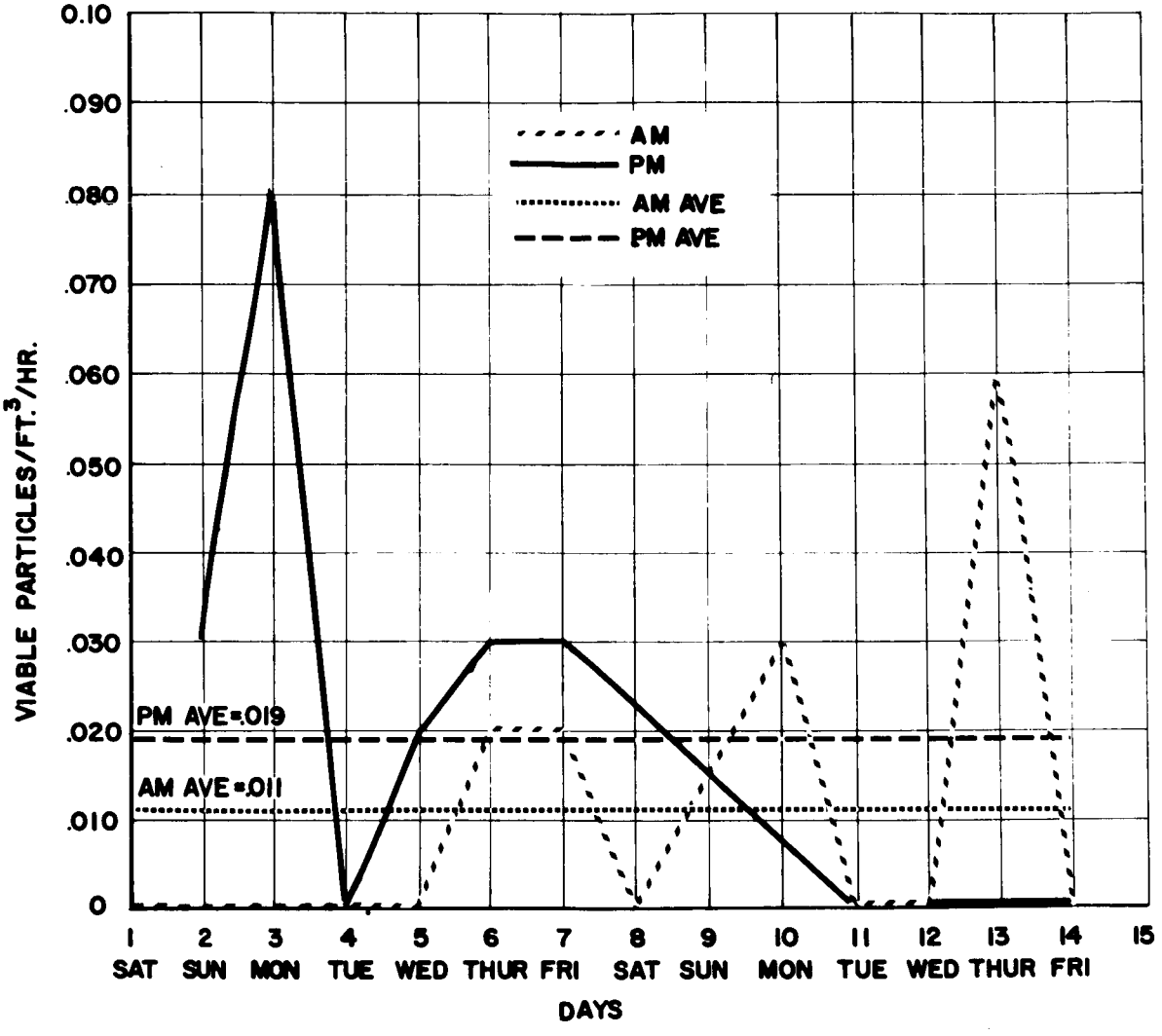
An average of the counts obtained in the six assembly phases (Table 4) indicates that the spacecraft had an average of 8060 viable organisms per square foot "before" decontamination, and 201 viable organisms per square foot "after" decontamination. The total spacecraft (500 square feet of surface area) would have a population of 4.03×10^6 viable organisms "before" decontamination and 1.00×10^5 after decontamination.

Figures 1, 2, and 3 illustrate the levels of microbiological contamination detected by a Reyneir sampler in the air of the two clean



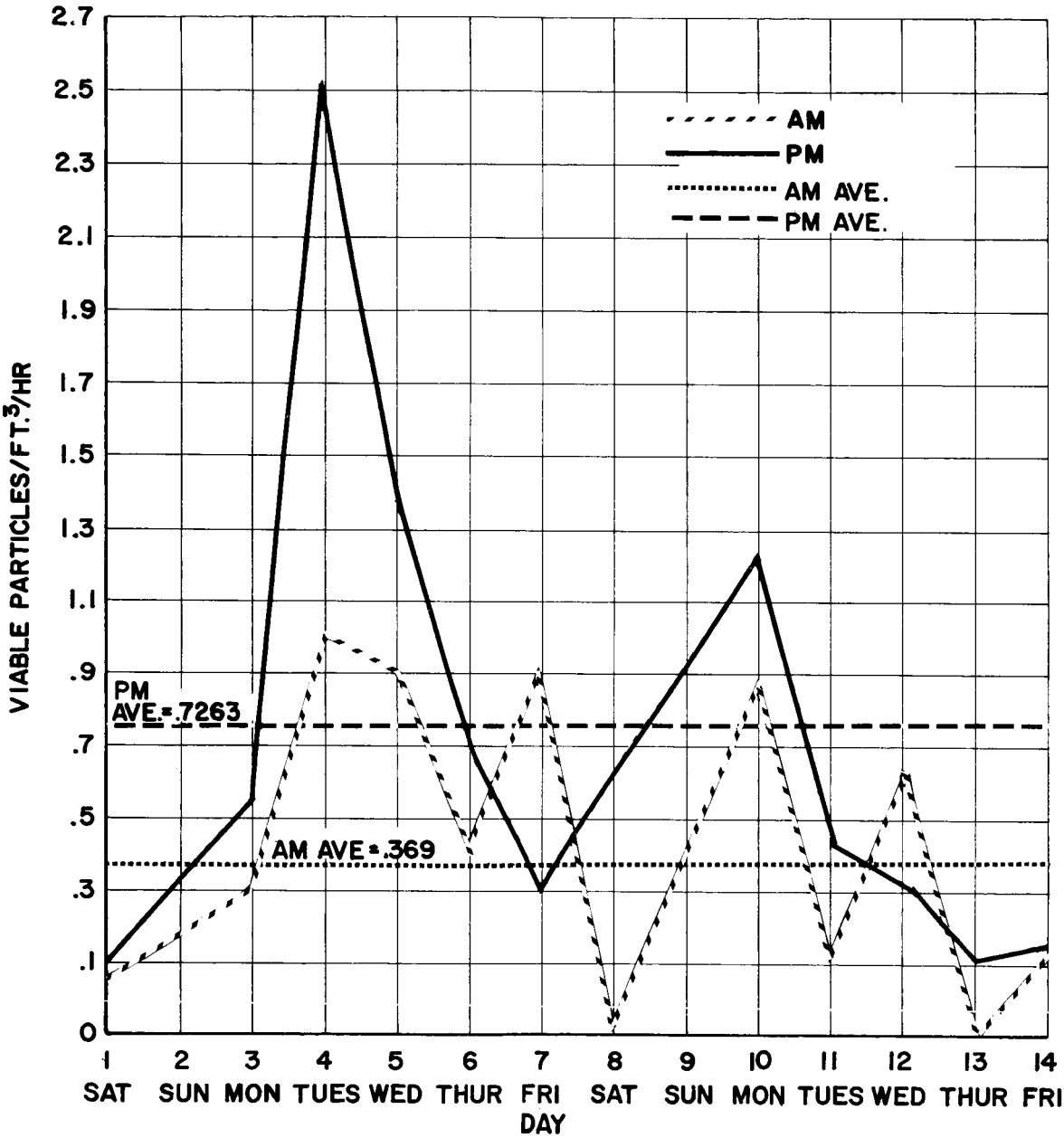
See Table 8.

Figure 1. Microbial Contamination in the Air of a Laminar Crossflow Clean Room



See Table 8.

Figure 2. Microbial Contamination in the Air of a Laminar Downflow Clean Room



See Table 8.

Figure 3. Microbial Contamination in the Air of the Potting Room

rooms and the potting room, showing counts obtained in the morning and afternoon, and the mean for the 14 days. Counts in the air of the crossflow room (Figure 1) ranged from 0 to 1.55 viable particles per cubic foot of air sampled per hour. Without exception, the points above the mean in Figure 1 represent times when the crossflow room was occupied. Contamination in the air of the crossflow room was not detected until the fourth day (Tuesday) which was the first day the room was occupied. Assembly and decontamination of the spacecraft began on this day.

Microbial contamination in the air of the downflow room (Figure 2) was an order of magnitude lower than that detected in the crossflow room; counts ranged from 0 to 0.08 viable particles per cubic foot of air sampled per hour. There was no significant increase in the contamination of the air when the downflow room was occupied.

Figure 3 presents the level of contamination detected in the air of the potting room. Counts were quite variable, ranging from 0 to 2.5 viable particles per cubic foot of air sampled per hour. All the high points in Figure 3 occurred when the room was occupied. The man who worked in the room went in and out frequently, so that the room was not always occupied for the entire duration of the sample.

Table 5 gives mean (average) counts from the air of the 3 rooms over a 14-day period (morning and afternoon). The potting room had a mean count 34 to 38 times greater than that detected in the downflow room, but only 2 to 3 times greater than that of the crossflow room. The mean was higher in the afternoon in all three rooms.

Table 5

Mean Viable Particle Count from Air of 3 Types of Rooms

	viable particles/ft^3/hr*			
	AM		PM	
Room	Mean	Range	Mean	Range
Downflow	0.011	0-0.06	0.019	0-0.08
Crossflow	0.114	0-0.38	0.286	0-1.55
Potting	0.369	0-1.04	0.726	0.11-2.5

*Reyneir slit sampler

It should not be inferred that the crossflow room was not much "cleaner" than the potting room. The air sampler, placed downstream of the spacecraft and personnel, reflects the contamination shed upstream of it. The relatively low count in the crossflow room, even downstream, points out the value of a laminar flow "clean" room over a conventional clean room.

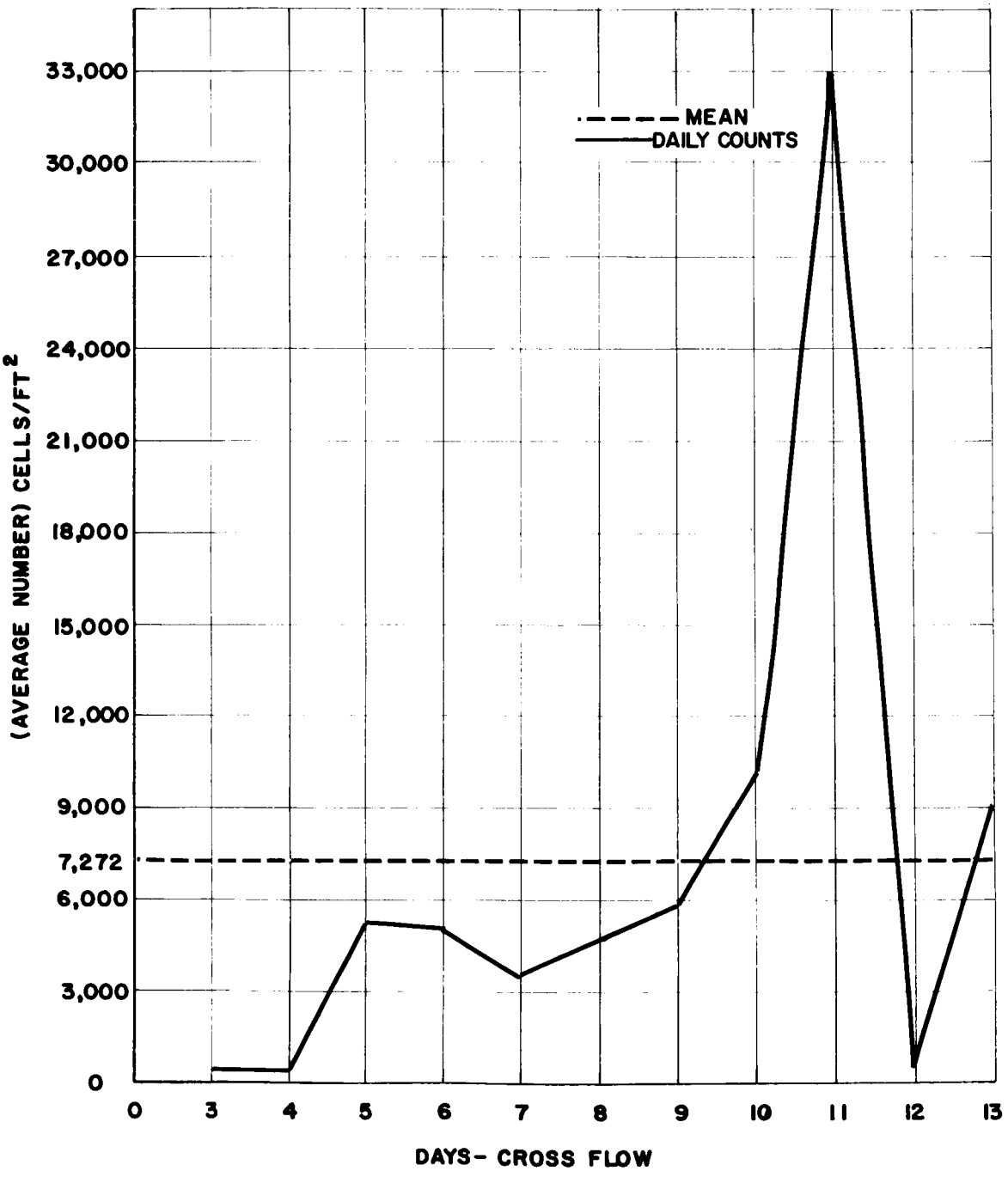
Figures 4, 5, and 6 illustrate the microbiological fallout detected on stainless steel strips exposed in the crossflow room, downflow room, and potting room respectively. The mean fallout is also depicted.

The viable microbial fallout in the crossflow room (Figure 4) increased gradually through the eleventh day from 432 organisms per square foot to 32,760 organisms per square foot. Activity on the eleventh day was unusual in that the room was occupied by three to five persons while electronic testing was performed, instead of the usual one to three.

Microbial fallout in the downflow room (Figure 5) ranged from 0 to 1,512 organisms per square foot. Except for the fifth day, which coincided with sampling of the flight spacecraft "before" decontamination, viable fallout remained within a relatively narrow range (0 to 216 organisms per square foot). Although the room was occupied every day, the greatest prolonged activity occurred on the fifth, eighth, and ninth days. The downward trend of the curve, from the ninth day to the thirteenth and last day, probably reflects both a slackening of activity and die-off of vegetative cells.

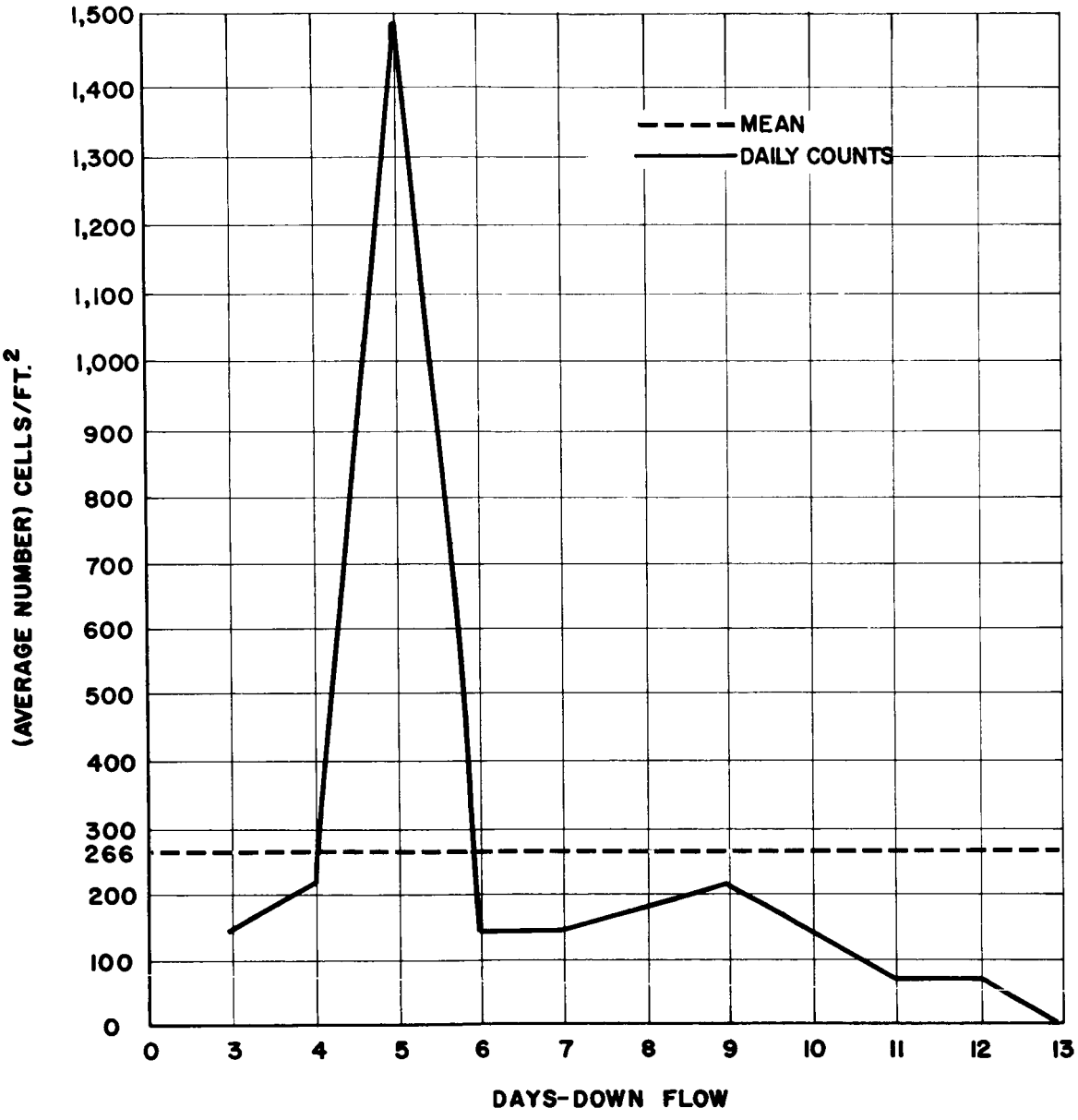
Microbial fallout on stainless steel strips in the potting room (Figure 6) ranged from 3096 to 49,680 viable organisms per square foot. The fallout increased gradually from the fourth day through the thirteenth day, except for the low counts detected on the eleventh and twelfth days. The high count detected on the third day may reflect violation of the strips sampled, by cleaning personnel.

Table 6 lists the mean microbial fallout shown in Figures 4, 5, and 6. The mean fallout in the crossflow room and potting room was 27 and 73 times greater, respectively, then the mean fallout detected in the downflow room. The difference between the crossflow room and the potting room was not as great, but it should be pointed out that the stainless steel strips in the crossflow room were placed downstream of the spacecraft and personnel.



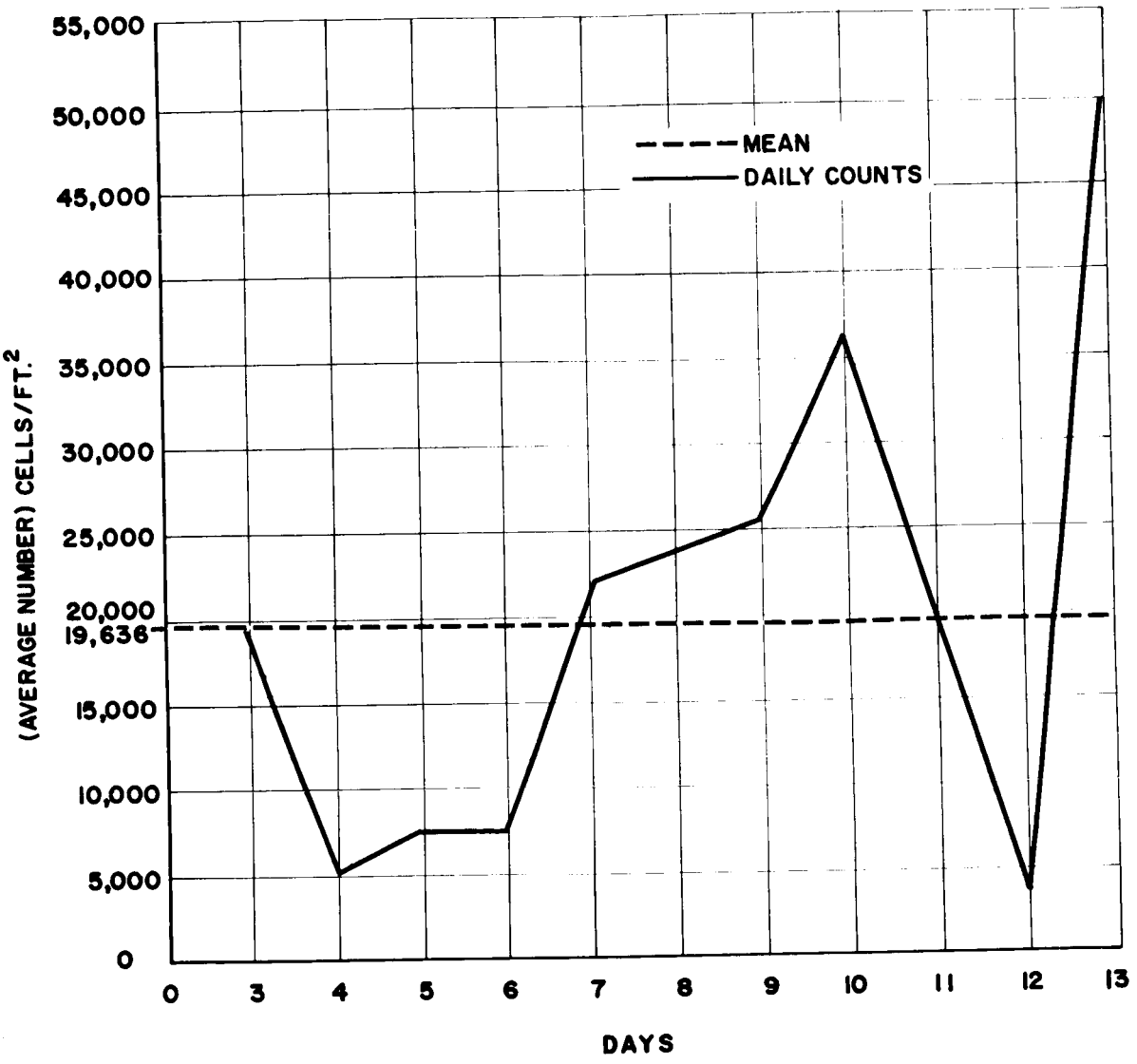
See Table 9.

Figure 4. Microbial Fallout on Stainless Steel Strips Exposed in a Laminar Crossflow Clean Room



See Table 9.

Figure 5. Microbial Fallout on Stainless Steel Strips Exposed in a Laminar Downflow Clean Room



See Table 9.

Figure 6. Microbial Fallout on Stainless Steel Strips Exposed in a Potting Room

Table 6

Mean Microbial Fallout on Stainless Steel Strips

	viable organisms per square foot	
Room	Mean*	Range
Downflow	266	0-1512
Crossflow	7272	360-32,760
Potting	19,636	3,096-49,680

*13-day average

Figures 7, 8, and 9 illustrate the microbiological fallout on tryptic soy agar plates after a 20-minute exposure. The mean viable fallout shown in Figures 7, 8, and 9 also appears in Table 7.

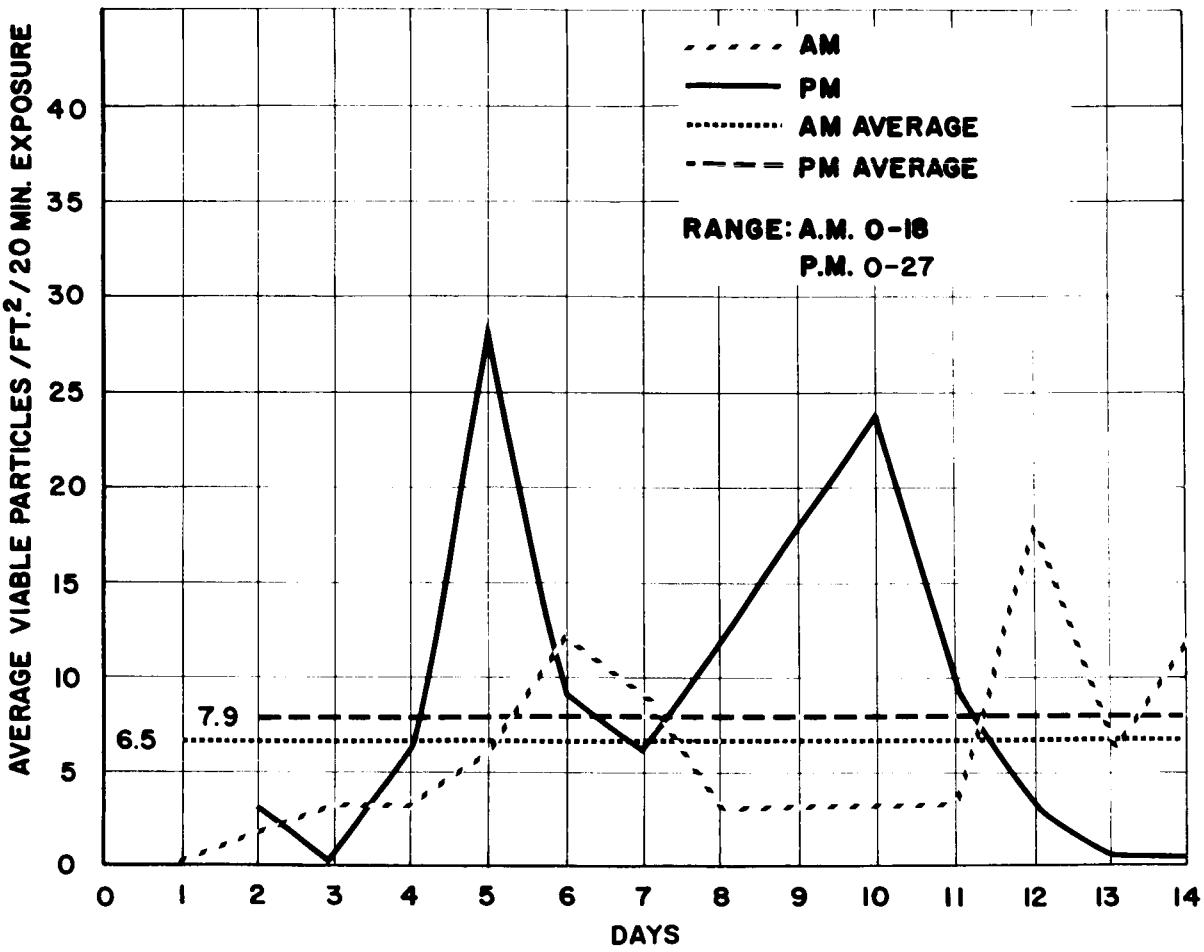
Table 7 shows that no great difference between the two laminar clean rooms was detected by the settling-plate technique. This technique did, however, demonstrate that the viable fallout in the potting room was a great deal higher than that in either of the laminar flow clean rooms. This points out quite well the value of a laminar flow clean room.

Table 7

Mean Microbial Fallout on Tryptic Soy Agar Plates

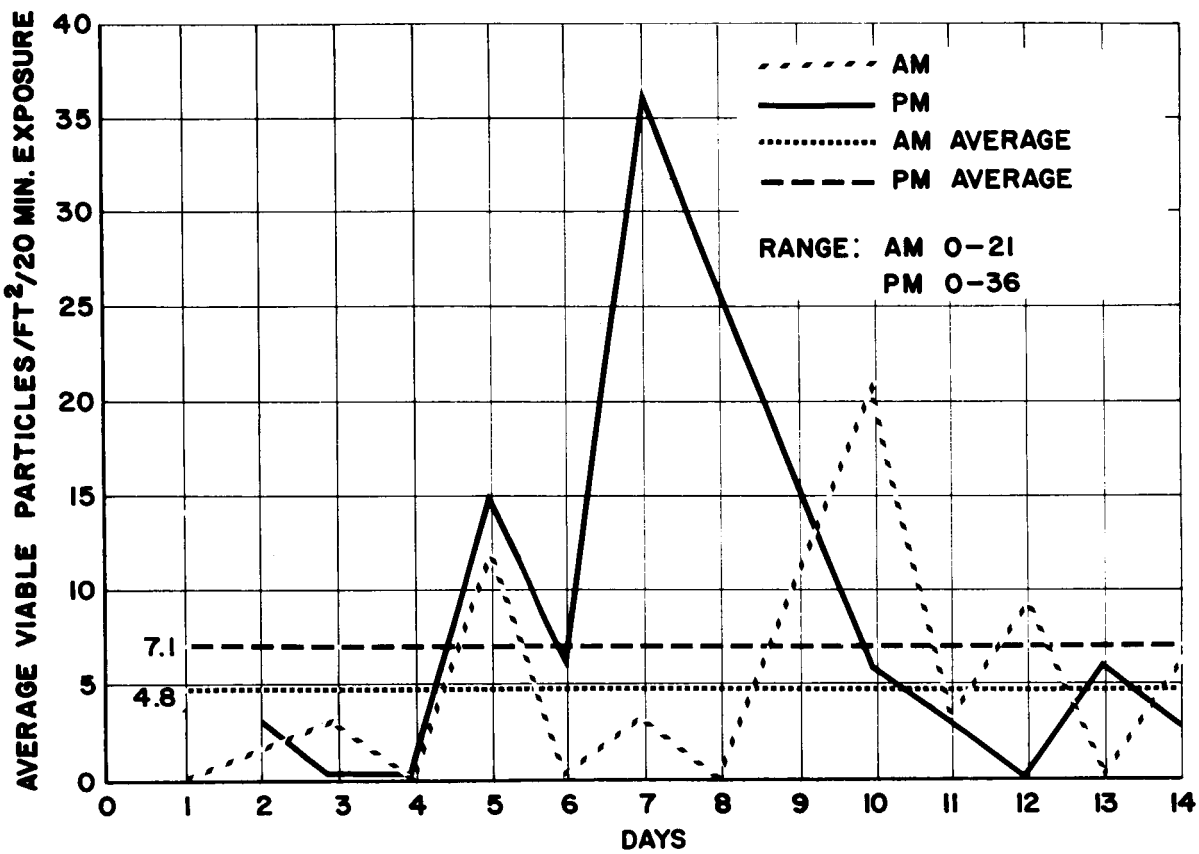
	viable particles/ft/20 min. exposure			
	AM		PM	
Room	Mean*	Range	Mean*	Range
Downflow	4.8	0-21	7.1	0-36
Crossflow	6.5	0-18	8.0	0-27
Potting	58.0	9-165	54.0	9-102

*14-day average



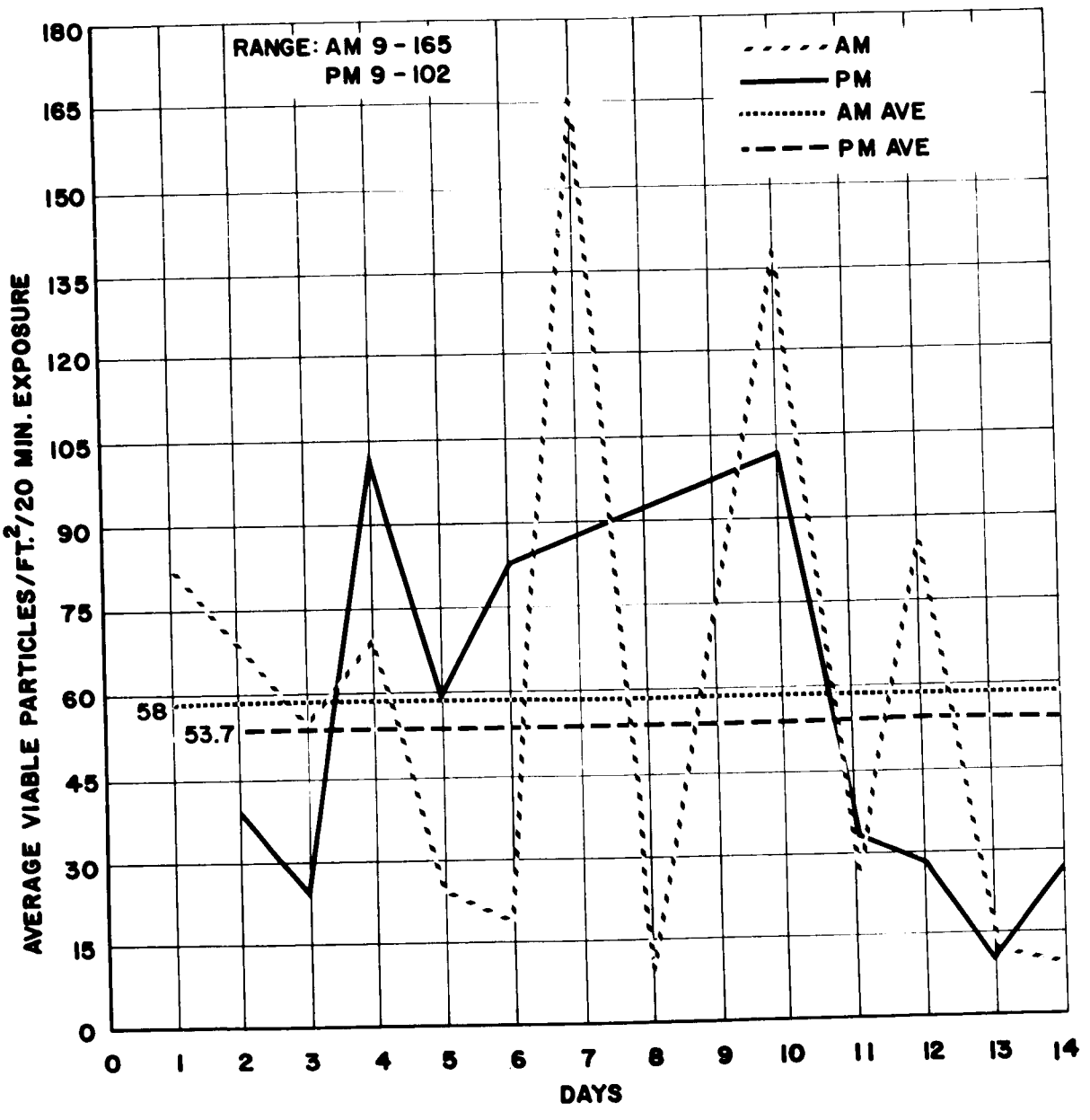
See Table 10.

Figure 7. Microbial Fallout on Tryptic Soy Agar Plates Exposed in a Laminar Crossflow Clean Room



See Table 10.

Figure 8. Microbial Fallout on Tryptic Soy Agar Plates Exposed in a Laminar Downflow Clean Room



See Table 10.

Figure 9. Microbial Fallout on Tryptic Soy Agar Plates in the Potting Room

DISCUSSION

The earlier estimation (Reference 1) that the total spacecraft at the time of launch would have approximately 1×10^5 organisms "after" decontamination still holds true. It was also estimated that the total microbial burden of the spacecraft would be approximately 1×10^7 organisms "before" decontamination.

The planned sterilization of the thermal blankets for assembly phase seven will reduce the microbiological contamination by more than half, because the multilayered thermal blankets alone have a total surface area of 300 ft^2. The estimated total burden will then be in the order of 4×10^4 organisms per spacecraft at time of launch.

Because of the temperature extremes and extreme vacuum that the spacecraft will encounter while orbiting the moon, as well as the long time factor before the spacecraft loses its orbit and impacts on the surface of the moon, it might also be estimated that the microbial population will be reduced by another one or two orders of magnitude.

Air sampling during the sixth assembly phase shows that the clean rooms had an extremely low level of microbial contamination. The results increase our confidence that the surfaces of the spacecraft were not recontaminated significantly during assembly.

Although the stainless steel strip method has merit and is a valuable tool for the accumulation of microbial fallout over long periods of time, variations in the counts obtained are probably inherent in the sampling device. First, a small number of strips are picked up and assayed, and estimates are based on extremely low colony counts not normally considered in conventional bacteriology. Second, the environment of laminar flow clean rooms is dynamic, so that fallout is not uniform. A clump of cells entrained in a dust particle or flake of skin may fall on one or two strips, which would increase the counts and account for high points on a graph.

Although the fallout on stainless steel strips was somewhat variable in the crossflow room and the potting room, the trend of the curve in Figures 4 and 6 was upward over a 13-day period. This upward trend was not apparent in the downflow room (Figure 5). A low point was reached in all three rooms (Figures 4, 5, and 6) around the twelfth or thirteenth day, which might be accounted for by die-off of vegetative cells.

Table 8

Microbial Contamination in the Air of Three Types of Room*

Day**		viable particles per cubic foot per hour					
		Downflow Room		Crossflow Room		Potting Room	
		AM	PM	AM	PM	AM	PM
1	Sat	0	--	0	--	0.08	--
2	Sun	--	0.03	--	0	--	0.32
3	Mon	0	0.08	0	0	0.3	0.54
4	Tues	0	0	0.35	0.27	1.04	2.5
5	Wed	0	0.02	0.24	0.56	0.91	1.39
6	Thurs	0.02	0.03	0.14	0.06	0.4	0.7
7	Fri	0.02	0.03	0	0.16	0.93	0.32
8	Sat	0	--	0	--	0.02	--
9	Sun	--	--	--	--	--	--
10	Mon	0.03	--	0.22	1.55	0.88	1.22
11	Tues	0	0	0.06	0.8	0.11	0.43
12	Wed	0	0	0.38	0.16	0.62	0.32
13	Thurs	0.06	0	0	0.02	0	0.11
14	Fri	0	0	0	0	0.14	0.14

*Reyneir sampler Table corresponds to Figures 1, 2, and 3. (--) No data

**April 30 - May 13, 1966.

Table 9

Microbial Fallout on Stainless Steel Strips
Exposed in Three Types of Room

Day*		viable particles per square foot		
		Crossflow Room	Downflow Room	Potting Room
3	Mon	432	144	19,080
4	Tues	360	216	5,256
5	Wed	5,256	1,512	7,798
6	Thurs	5,040	144	7,704
7	Fri	3,528	144	21,960
8	Sat	--	--	--
9	Sun	5,688	216	25,488
10	Mon	10,296	144	36,720
11	Tues	32,760	72	19,584
12	Wed	432	72	3,096
13	Thurs	8,928	0	49,680

Table corresponds to Figures 4, 5, and 6. (--) No data
*May 2 - May 12, 1966.

Table 10

Microbial Fallout on Tryptic Soy Agar
Plates Exposed in Three Types of Rooms

		viable particles per square foot per 20-minute exposure					
		Crossflow Room		Downflow Room		Potting Room	
Day*		AM	PM	AM	PM	AM	PM
1	Sat	0	--	0	--	84	--
2	Sun	--	3	--	3	--	39
3	Mon	3	0	3	0	54	24
4	Tues	3	6	0	0	69	102
5	Wed	6	27	12	15	24	57
6	Thurs	12	9	0	6	18	84
7	Fri	9	6	3	36	165	87
8	Sat	3	--	0	--	9	--
9	Sun	--	--	--	--	--	--
10	Mon	3	24	21	6	138	102
11	Tues	3	9	3	3	27	33
12	Wed	18	3	9	0	87	27
13	Thurs	6	0	0	6	12	9
14	Fri	12	0	6	3	9	27

Table corresponds to Figures 7, 8, and 9. (--) No data
*April 30 - May 13, 1966.

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